THE ADRENAL GLAND IN NEW-BORN MAMMALS.

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(Plate xi; twenty Text-figures.)

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For this work the adrenals of the following species were examined: (1) Two new-born specimens of *Equus zebra*; (2) one new-born *Ursus maritimus* (Polar Bear); (3) one young specimen (a few weeks old) of *Arctocephalus doriferus* (Seal); (4) one late fœtus of *Delphinus delphus* (Dolphin); (5) one ten-inch pouch-embryo of *Dendrolagus bennettianus* (Tree Kangaroo); (6) one ten-inch pouch-embryo of *Macropus giganteus* (Kangaroo); (7) one four-inch female and one five-inch male pouch-embryo of *Macropus ruficollis*; (8) one young female specimen of *Macropus agilis*.

The two young zebras were obtained by courtesy of the Zoo authorities in Western Australia, and other glands described were obtained from preserved specimens in the possession of the Australian and National Museums, and the Western Australian Museum.

As a result of the imperfect fixation of the glands in the museum specimens, accurate details regarding the cells could not be given, but it was possible to distinguish the various zones and to make some degree of comparison with other forms.

Fixation and Staining.

The adrenals of the zebras were removed and divided into various sections. One portion was placed in 10% formalin for two days, then washed, imbedded, and sectioned. The sections were stained by the haemalum-eosin method.

Other sections of the zebra adrenal were treated in various ways. A portion was fixed in formalin for 24 hours; frozen sections were treated with Scharlach R and Sudan III to demonstrate fats and lipoids, and then mounted in glycerine jelly. Another portion was treated with osmium tetroxide (osmic acid) to test for the presence of unsaturated fat. In addition a portion of one gland was fixed in a mixture of 3% potassium bichromate 2 parts, 1% chromic acid 2 parts, and 2% osmic acid 1 part, for twenty-four hours, then placed in 2% osmic acid for four days at 30° C. (Kolatchev's method); the resulting sections demonstrated the Golgi apparatus.

For mitochondria another piece of gland was placed in the above fixative for twenty-four hours, washed, mordanted for six days in 3% potassium bichromate, washed, and sectioned. The sections were stained by the classical acid-fuchsin-picric-acid method.

The Staining of the Vitamin C.

Of two further pieces of gland, one was placed in the present author's modification of Giroud and Leblond's acetic acid silver nitrate mixture for two hours in the dark and kept at room temperature. Another piece was placed in acetic acid gold chloride for a similar time and under identical conditions. The composition of the silver nitrate solution was 2% silver nitrate with 5 c.c. of glacial acetic acid added to each one hundred cubic centimetres of the silver nitrate solution. The gold chloride solution was of a similar strength. The results obtained by these two methods were identical.

Treatment of Museum Specimens.

The adrenals removed from museum specimens were spirit-preserved. They were washed in distilled water for 48 hours, treated with 10% formalin for a further 48 hours, washed with distilled water for 24 hours, and were then dehydrated and sectioned. The sections were stained by the haemalum-eosin method as for the fresh adrenals.

Previous Work.

Hill (1930) described the microscopic and macroscopic appearances of the adrenal glands in young and adult specimens of Primates, Carnivores and Ungulates. He found that a foetal cortex was present in those specimens of young Primates which he examined, and stated that in young Carnivores and Ungulates the main mass of the gland was composed of cells which were morphologically similar to those of the "foetal cortex" of Primates.

The "foetal cortex" might be called an enlarged boundary zone between cortex and medulla. It is present in the human foetus and in the very young of both sexes, and was discovered in 1911 by Elliott and Armour. Starkel and Wegrzynowsky, Kern, and Thomas published on the same subject at about the same time. This cortical zone is not found in any other mammals lower than the Primates, and its absence from the human foetal adrenal in cases of anencephaly has led certain authors to claim a relationship between brain development and the presence of the "foetal cortex". It is of interest in this connection that the "foetal cortex" is only present in the adrenal gland of such members of the mammalian order as show a distinct advance in cerebral development over the members of the other orders.

The presence of large quantities of lipoid in both brain and adrenal gland may be a factor of some significance, but the "foetal cortex" is sometimes almost devoid of lipoid or may contain only relatively small amounts. Cooper (1925) mentions that at times the cortical cells may show marked reduction of lipoid, but she points out that as lipoidal material is apparently concerned with the development of the central nervous system and the sexual gland, therefore "it seems reasonable to expect the suprarenal body, which is presumably an important source of lipoidal material, to produce large amounts of this substance at those periods of life when the development of the sexual organs and the nervous system is greatest, namely, at puberty. Ordinary histological examination of the gland appears to support this presumption . . ."

Hill has published a summary of the more important papers dealing with the development of the adrenal gland in various animals, e.g., Jackson, 1913; Donaldson, 1919; Dewitsky, 1912, on rats; Elliott and Tuckett, 1906, on guinea-pigs; Lucas-Keene and Hewer, 1927, on the human adrenal; and Howard-Miller, 1927, on the X zone of the adrenal in mice. The animals examined by Hill include new-born Carnivores (kittens, leopard cub, pups, bear cub), Ungulates (lambs, pigs, goats and foetal hippopotami and calves), and Primates (Pithecoid monkeys, Macaques and Lemurs).

The Adrenals of New-Born Specimens of Equus zebra.

The adrenals in both specimens were elongated, smooth, rounded bodies of a light colour and were in each case attached intimately to the antero-mesial borders of the corresponding kidneys (Fig. 1). The sizes were: Left adrenal, 12 mm. long, 3 mm. antero-posterior thickness, and 3·5 mm. wide. Left kidney, long axis 40 mm., width 25 mm., and dorso-ventral thickness 15 mm. The gland was attached to the kidney by a plentiful investment of connective tissue which anchored it firmly in position.

On section, the gland showed a thin outer rim of cells and a more voluminous medullary part. Just beneath the capsule was a striking zone of elongated dark-staining cells which were arranged side by side with the long axes parallel to the capsule, and these cells formed columns which bent round through 180 degrees at the capsule and returned to the main body of the cortex.

The major portion of the cortical rim was made up of large cells with big vesicular nuclei as described by Hill for other Ungulates. Hill, however, found, beneath the capsule, a zone of small, darkly-staining cells not oriented in any glomerular fashion, and he comments that even in a three-weeks-old pig he is not able to find any glomerular arrangement of these cells. The young zebra adrenal, then, is an exception to the Ungulates examined by Hill in that, even at birth, a definite zona glomerulosa is present.

Near the glomerular zone the cortical cells with vesicular nuclei have scattered among them cells of similar size but with much more chromophillic nuclei. As the central medullary portion is more closely approached, these cells gradually become fewer and fewer and, closer to the medullary portion, large cells with very vesicular, weakly-staining nuclei make their appearance. Thus in a stained section of the gland we obtain a gradation of shading from the darkly-staining glomerular region to the lightly-staining medullary portion. This is shown very well in Plate xi, fig. 1.

The cells of this cortical rim were not oriented in any particular direction. This differs from the arrangement in the adrenals of the animals examined by Hill, in which he found the cortical cells arranged in short columns radiating from the centre of the gland.

The boundary zone between the cortical rim and the medullary portion is rather well defined and, although tongues of cortical material penetrate into the medullary portion, they are well marked off from the central or medullary region of the gland.

Immediately within the cortical portion were groups of very large cells with a pale-staining cytoplasm and very large, somewhat vesicular nuclei. They were in some cases isolated, but where they occurred near the cortical rim they were aggregated into groups with the long axes of the cells parallel with one another and at right angles to the boundary line. They were separated from the cortical rim by a single layer of flattened fibroblasts. These cells constituted the true medullary cells (Fig. 3). In addition to these, there were numerous other cells of a smaller size with vesicular, but more intensely staining nuclei and light-staining cytoplasm. These were scattered through the medullary portion of the gland and were very similar in nature to cortical cells. In addition, large cells with a much more deeply staining nucleus were present and numerous cells similar to those described near the boundary zone were found scattered through the so-called medullary portion. Also, there was a goodly admixture of large well-formed fibroblast cells with typical oval nuclei. The so-called medulla of this gland is thus a mixture of cortical elements and medullary elements and the gland may be described as consisting mainly of cortex, as Hill has found for other Ungulates.

Hill regards the main mass of the cells in the Carnivore and Ungulate adrenals as being similar to those of the "foetal cortex" of Primate adrenals. The foetal cortex, however, is, as Hill mentions, a zone which subsequently degenerates and takes no part in the formation of the adult cortex. No signs of degeneration have been observed in the zebra adrenal by myself, or by Hill in the adrenals of non-Primate animals examined by him. In the human foetal cortex, however, degeneration products, in the form of numerous melanin granules, have been found to be present, as a number of authors have observed.

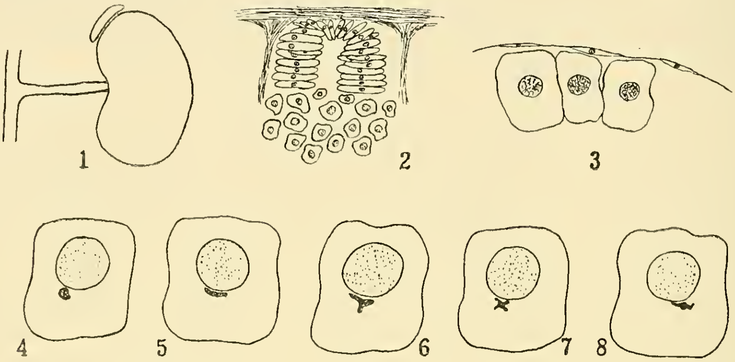

Fig. 1.—Relationship of the left adrenal to the left kidney in the new-born *Equus zebra*. × ½.

Fig. 2.—Arrangement of cells in the zona glomerulosa in *Equus zebra*. × 500.

Fig. 3.—Arrangement of medullary cells at boundary between cortex and medulla in *Equus zebra*. × 1,750.

Figs. 4, 5, 6, 7, 8.—Golgi apparatus in the cells of the adrenal of the new-born *Equus zebra*. Fig. 4, a cell from the cortical rim; Figs. 5 to 8, cells from the central portion of the gland.

In Ungulates, Hill has shown that the foetal cells modify directly into the cells of the adult adrenal without any degeneration. I have not been able to obtain a series of zebra adrenals to confirm this fact.

The young zebra adrenal showed a paucity of lipoidal material in nearly all of its cells. Unsaturated fat also was found to be very rare in most of them (osmium tetroxide test).

A number of cortical cells showed the presence of two or three globules of lipoid enclosed in a small area which stained intensely black with osmium tetroxide, thus indicating the presence of unsaturated fat. This was also observed in occasional cells of the adrenal of the flying Phalanger (*Petaurus breviceps*) and may indicate that one of the substances associated with the elaboration of the lipoid droplets may be unsaturated fat. The presence of grey granules in some of the cells indicated the presence of fat of a higher degree of saturation. Mitochondria were found to be present in all the cells and were observed to be typically granular and to be scattered amongst the lipoidal globules (where present), but there did not appear to be any association between them and the lipoid droplets. No filamentous mitochondria were observed in any of the cells. This result (granular mitochondria) is in accord with the work of the previous authors on the adrenal gland. Pleknik (1902) and Bonnamour (1902) have found granular fuchsinophilic material, which they regarded as mitochondria, in the cells of the adrenals of various animals. Mulon (1910) found rod-shaped mitochondria in the zona glomerulosa cells of the guinea-pig adrenal. Rogoff (1932) quotes Seecof as having found the mitochondria of the adrenal to occur most commonly in the form of minute rounded granules, taking the rod-shaped and filamentous form only very infrequently.

Golgi Apparatus.

All the cells of the zebra adrenal showed the presence of a Golgi apparatus. In the cells of the cortical rim it was of a very compact nature and appeared to be composed of a sphere with numerous internal anastomosing branches (Fig. 4). This was the only type of apparatus found in the outer region, and no signs of hypertrophy of the apparatus as described by the present author in the adrenals of other animals was observed. This is in agreement with the suggestion (Bourne, 1934) that the Golgi apparatus is associated with the production of the lipoid droplets in the cortical adrenal cells, for here, where there are very few lipoid droplets, the Golgi apparatus is quite small and appears inactive. In the central portion of the gland the Golgi material, in some cells, was of the form just described and in others it showed a greater variety of forms (Figs. 5–8). Although the shape of the apparatus varied in these cells, it may be seen that it was still compact and showed no signs of hypertrophy.

Vitamin C.

The material presumed to be vitamin C was found to be aggregated chiefly in the central part of the gland and in the inner part of the true cortex. The zona glomerulosa and the outer portion of the zona fasciculata gave no reaction with either of these techniques, so it is probable that reduced vitamin C is absent from this portion of the gland (Pl. xi, fig. 2). Giroud and Leblond (1934) have found the adrenals of adult rats and guinea-pigs examined by them to contain no vitamin C in the zona glomerulosa, but they found it to be present plentifully in the zona reticularis and the zona fasciculata. The form taken by the vitamin was identical in the two techniques, that is, finely granular, with a slight tendency towards perinuclear condensation. The granules were found to be solid, not hollow as in the adrenals of a human foetus examined by the author. The fact that the outer portion of the glands gave no reaction with silver nitrate is not absolute proof that the vitamin is absent from this region, as Harris (1933) has found that in some cases the regions which do not stain with silver nitrate actually contain varying quantities of the reversibly oxidized vitamin, but he considers a positive result with silver nitrate a definite indication of the presence of the vitamin.

Ursus maritimus.

The adrenals of a new-born polar bear were examined. The glands were found to occupy the antero-mesial borders of the corresponding kidneys and showed a regular oval shape in transverse section. The surfaces were quite smooth and were covered by a thick loosely-investing capsule. The relations of the glands with the corresponding kidneys are shown in figure 9.

In section (Fig. 10) the glands did not appear to possess a definite medulla. There was an outer peripheral, denser, rim of cells and the main body of the gland appeared to be composed of mixed cortical and medullary elements. The peripheral cells showed in parts the structure of a zona glomerulosa.

Arctocephalus doriferus.

A young specimen a few weeks old was examined. The glands were elongated, and possessed a thick but loosely-investing capsule. The surfaces of the two adrenals were smooth. Both glands were situated on the anteromesial borders of the corresponding kidneys to which they were firmly attached (Fig. 13). The capsule sent practically no trabeculae into the interior of the gland. The cortex was fasciculated and there was a small zona glomerulosa and a small zona reticularis (Fig. 14). Thus, even at this early age in the seal, the typical adult structure of the gland is present. In 13-day-old members of the Carnivora vera, Hill found a zona glomerulosa and an incipient zona fasciculata to be present.

Fig. 9.—Relation of the adrenals to the kidneys in *Ursus maritimus* (new-born). × ¼.

Fig. 10.—The thin cortical rim surrounding a central portion containing mixed medullary and cortical elements. Portion of the cortical rim may be seen to possess a zona glomerulosa. × 5.

Fig. 11.—Position of adrenals in *Delphinus delphus.* × ¼.

Fig. 12.—Thin cortical rim with well defined zona glomerulosa. × 4.

Fig. 13.—*Arctocephalus doriferus.* Position of adrenals. × ¼.

Fig. 14.—Adrenal of young *Arctocephalus doriferus*, showing the three typically differentiated zones of the adult gland. × 5.

Fig. 15.—*Macropus ruficollis.* Position of adrenals. × ¼.

Fig. 16.—*Macropus ruficollis.* Showing strands of cortical material penetrating the medulla. Pouch-embryo. × 4.

Fig. 17.—*Macropus giganteus.* Position of adrenals. × ¼.

Fig. 18.—*Macropus giganteus.* Partly differentiated adrenal of pouch-embryo, showing zona glomerulosa. × 4.

Fig. 19.—*Dendrolagus bennettianus.* Position of adrenals. × ¼.

Fig. 20.—*Dendrolagus bennettianus.* Adrenal of pouch-embryo, showing slightly fasciculated cortical rim. × 3.

Delphinus delphus.

A late foetus was examined. Each gland was placed cranially to the corresponding kidney and was intimately attached to the dorsal body-wall (Fig. 11). The surface of the gland was smooth, though the shape was irregular. The cortex was rather difficult to distinguish from the medulla, and there was a typical thin rim of cells surrounding the gland, which stained much more intensely than the deeper portions. A zona glomerulosa was present also in this animal. The central region, as in the previous animals examined, was composed of mixed cortical and medullary elements (Fig. 12).

The pouch-young of three Marsupials of the family Macropidae were examined. In all three the adrenals were situated typically on the antero-mesial borders of the corresponding kidneys, and their surfaces were quite smooth (Figs. 15, 17, 19).

Dendrolagus bennettianus.

The adult gland of this Marsupial possessed a well-defined broad cortex, together with a rather imperfectly formed zona glomerulosa. In a 10-inch male pouch-embryo (Fig. 20), the cortex was in the form of a thin rim which possessed a slight degree of fasciculation and an indistinct zona glomerulosa. A number of trabeculae extended through the cortex and cut off portions of this region of the gland into separate islets of cells. The central portion of the gland contained both cortical and medullary elements as in the young Eutherian adrenal. The central cells were irregularly arranged.

Macropus giganteus.

A 10-inch pouch-embryo showed an adrenal with but a thin rim of cortex which was, however, definitely fasciculated and possessed a well-defined zona glomerulosa. The central portion again contained mixed cortical and medullary elements (Fig. 18).

Macropus ruficollis.

Four- and five-inch female and male pouch-embryos were examined. The cortex was composed of a rim of completely undifferentiated cells irregularly arranged. Some of the cells in this region, however, were arranged in strands and some of these strands entered the medulla to form a network, in the meshes of which the medullary cells lay in groups. This condition is quite primitive (Fig. 16).

Macropus agilis.

A young female specimen possessed adrenals which showed a thin differentiated cortex and a well-defined voluminous medulla which contained chiefly medullary and only a few occasional cortical cells.

Summary.

Hill's work on the adrenal glands of young Carnivora and Ungulates has been supported by the examination of other members of the same orders. In addition, it appears that the adrenals of members of the Metatheria Macropidae also pass through a stage comparable with that of the adrenals of the non-Primate Eutherian Mammal.

A similar stage may be seen in the adrenals of the Porpoise (*Delphinus delphus*). A young seal (*Arctocephalus doriferus*), however, possessed an adrenal in which a definite differentiated cortex was present, thus constituting a difference from the adrenals of members of the Carnivora vera, examined by Hill.

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EXPLANATION OF PLATE XI.

Figure 1.—Section through adrenal of new-born *Equus zebra*. The thin rim of cortex with definite zona glomerulosa may be easily distinguished. × 80.

Figure 2.—Vitamin C in the adrenal gland of the new-born *Equus zebra*. The absence of the stain from the outer portion of the cortical rim may be easily noted. × 80. (Gold chloride method.)